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Monophyly of Eusocial Wasps (Hymenoptera: Vespidae): Molecules and Morphology Tell Opposing Histories

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Introduction

The evolution of insect societies, as seen in eusocial ants, bees and wasps, is one of the most exceptional biological phenomena to ever occur. Eusociality involves a phenotypic decoupling of workers and queens (i.e. the incipience of castes) where some individuals forfeit direct reproductive success and instead cooperatively rear the brood of another (i.e. altruism). Social insects comprise just 2% of all insect species, but over half the total biomass of insects [1]. Although eusociality is related to ecological dominance, presumably the emergence of social life is contingent upon evolutionarily 'expensive' precursor traits. Although not for bees, subsociality is generally recognized as a salient precursor for eusociality in vespid wasps [2]. Subsociality involves progressive provisioning (offspring fed in daily increments, not in mass), which prolongs offspring dependency. Vespidae is comprised of over 5000 wasp species, of which approximately 1100 are eusocial [3]. In primitively eusocial wasps worker status may be temporary; all females are potential queens (e.g. independent-founding Polistinae; Stenogastrinae). Highly eusocial wasps possess a permanent worker caste (e.g. swarm-founding Polistinae; Vespinae).

Phylogenies provide insight into the ancestral states and environmental contexts that subserve worker/queen decoupling, and thus are the framework for understanding origins of eusociality. We present a thorough phylogenetic analysis of the Vespidae, utilizing phenotypic and molecular data of previous studies [3,4] in conjunction with newly acquired data, in an effort to explain how solitary ancestors may have crossed the threshold of eusociality. Previous studies show conflicting phylogenies for Vespidae and differ in their conclusions regarding whether eusociality has evolved once or twice in these wasps [3,4]. Here we explore the primary drivers of phylogenetic incongruence complicating reconstruction of the Vespidae phylogeny.

Methods

Simultaneous analysis of phenotypic characters (269 morphological and 66 behavioral) and molecular data (COI, 28S, 16S, 12S) for 74 taxa was performed to clarify the subfamily relationships of the Vespidae. Sequence alignments were implemented within Codon-code aligner v.3.7.1.2 using the ClustalW option and subsequently vetted by-eye. All COI sites were included in the final alignment because an underlying protein code guides the vetting process. However, many sites of rDNA were excluded. Encryption of indel events is unfeasible for regions of rDNA sequences that are hyper-variable

(i.e. functionally unconstrained). The number of possible multiple sequence alignments corresponding to these indel-rich regions are legion a mere ten sequences with a length of 5 base pairs each yields 1.35 x 1038 possible alignments [5] making the search for an exclusive alignment of minimum cost problematic. Thus, these regions were ignored and only the most confidently aligned nucleotide sites are included as input for phylogenetic analyses. The final concatenated alignment was comprised of 4051 base pairs, of which 1056 were parsimony informative, and 335 phenotypic characters. The phenotypic characters are adopted from previous work [3]. Gaps were consistently treated as missing data and the ingroup was forcefully constrained.

In addition to simultaneous analysis of all data, we performed analyses based solely on molecular data, and on all data except behavioral. To account for incongruence brought on by different analytical methods, we employed Maximum Likelihood, Bayesian Inference and Maximum Parsimony for all data-inclusion schemes. Model testing was implemented in jModelTest 2 [6] to determine the best-fit model for each partition of molecular data. A separate unlinked model was applied to each COI codon because this partitioning scheme returned the lowest Akaike Information Criterion score. The third codon position was included despite potential homoplasy because no major differences were observed between the COI gene trees retrieved from utilization of only the first two codon positions and all codon positions (not shown).

To find the most parsimonious trees, a new technology search strategy of 1000 random additions, each with 40 rounds of parsimony ratchet, 20 rounds of tree drifting and 30 rounds of fusing was implemented within TNT [7]. Phenotypic characters were treated as non-additive in our analyses. ML analyses were performed in GARLI [8]. The models implemented were: GTR+I+G for the first codon of COI, TVM+I+G for the second codon of COI, TPM1uf+I+G for the third codon of COI, GTR+I+G for 28S, GTR+I+G for 16S and HKY+I+G for 12S. An Mkv unordered model was specified to the phenotypic characters. Bayesian analyses were performed with MrBayes 3.2 [9]. Priors were left flat. All DNA partitions were assigned the GTR+I+G model, except 12S was assigned the simpler HKY+I+G model. Phenotypic data was assigned an Mkv model with gamma rate heterogeneity. In both ML and Bayesian analyses, models were unlinked and allowed to evolve with independent rates of evolution. A relative burn-in of 25% was set and 2 runs with 4 chains each were performed. All analyses were allocated 3 million generations and reached a standard deviation of split frequencies below 0.01.

Results

Simultaneous analysis of all evidence supports a (Stenogastrinae + (Polistinae + Vespinae)) clade and thus a single origin of eusociality (Fig. 1A, D, G). Phylogenies derived strictly from molecular evidence consistently support a dual origin of eusociality (Fig. 1C, F, I). Our results more-or-less support the novel tribal relations of Polistinae recently proposed [3] as (Ropalidiini + (Mischocyttarini + (Polistini + Epiponini))), but the strength of support for a Polistini + Epiponini clade diminishes with the addition of phenotypic data (Fig. 1). Both the nuclear and mitochondrial DNA trees did not support monophyly of the eusocial wasps (not shown).

Discussion and Conclusions

Our reanalysis shows that previous studies recovering diphyly (two distant clades) of eusocial wasps [4] may withstand the effects of low taxon sampling, suboptimal alignments and a two-step approach causing phylogenetic error; we achieved similar topologies using three-fold more terminal ingroups and a different suite of loci. Additionally, our two-step regime retrieved a similar maximum parsimony topology (Fig.1G) as a study that employed one-step direct optimization [3]. Our findings elicit an original perspective concerning Vespidae systematics: There are biological processes causing phylogenetic conflict, which are presumably driving conflict more prominently than methodological drivers.

Our results qualitatively show that a portion of phylogenetic incongruence is attributable to method choice, but that the primary driver of incongruence is data type inclusion that is, the topology produced changes when phenotypic data is included (Fig.1). We conclude that vespid subfamily relationships are muddled by the fact

that phenotypic and molecular evidence are at odds. One or several of the following processes may be responsible for phylogenetic incongruence: phenotypic and/or molecular saturation; phenotypic and/or molecular adaptive convergence; paralogy; introgression; and incomplete lineage sorting. Addressing the significance of biological processes driving phylogenetic conflict in vespids will require analyses of vast genomic data.

We advocate topologies retrieved from simultaneous analysis of all available evidence. It can be inferred that facultative nest sharing, cooperative rearing and a rudimentary worker phenotype evolved concurrently under a context of simultaneous progressive provisioning (SPP) before divergence of Stenogastrinae and Polistinae + Vespinae. We posit that the emergence of SPP is pivotal to the incipience of a temporary worker caste. The energetic costs of brood care inhibit ovarian development [10]. If the queen transfers the burden of larval care onto emerging daughters, then the latter will be coerced into becoming temporary workers. Furthermore, the sting venom of progressively provisioning wasps is free to exapt a novel function and in eusocial wasps has specialized as an anti-vertebrate defense due to its dissociation from prey handling. Anti-vertebrate venom likely evolved after eusocial habits and is thus important in the maintenance, rather than origin, of eusociality. The capacity for allomaternal care (adopting orphaned larvae) has evolved independently in multiple lineages of vespids [2], indicating that vespid wasps have a propensity for it. SPP may have provided a novel social context that exploited and altered pre-existing behavioral flexibility, resulting in exaptation of allomaternal care into cooperative allomaternal care and the emergence of a rudimentary worker phenotype via transference of strenuous larval care onto emerging daughters. Also, the phylogeny infers at least four independent origins of swarm-founding behavior [10] (Provespa (Vespinae); Epiponini; and twice within Ropalidiini), implying that a permanent worker caste has evolved multiple times.

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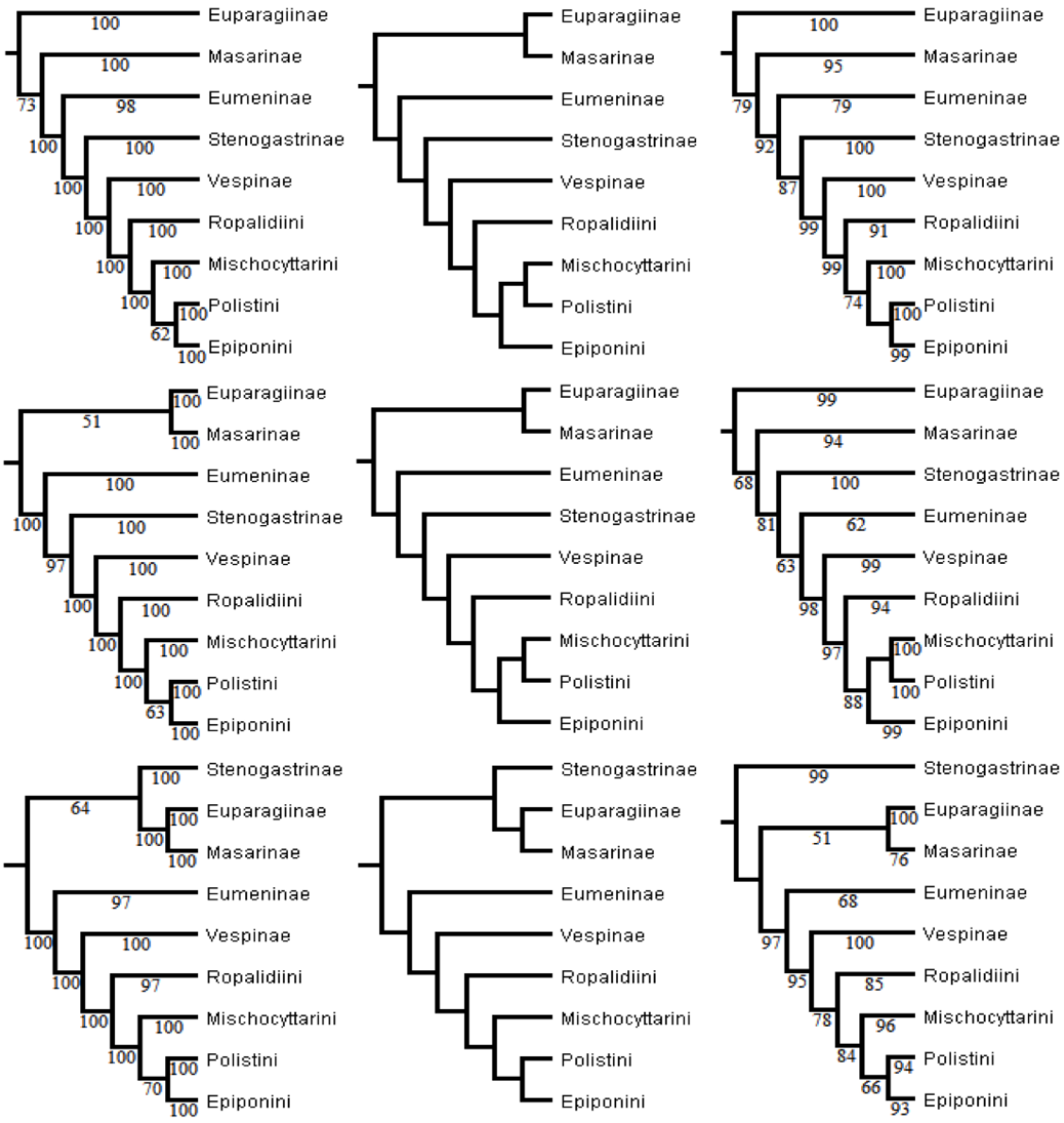

Figure 1:
Condensed phylogenies of the Vespidae obtained in the current study via Bayesian Inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP). **A, B, C**: Bayesian consensus tree resulting from analysis of: **(C)** all molecular characters; **(B)** all molecular and morphological characters; **(A)** all molecular, morphological and behavioral characters. **D, E, F**: ML tree resulting from analysis of: **(F)** all molecular characters; **(E)** all molecular and morphological characters; **(D)** all molecular, morphological and behavioral characters. **G, H, I**: Strict consensus MP tree, from analysis under equal weights of: **(I)** all molecular characters; **(H)** all molecular and morphological characters; **(G)** all molecular, morphological and behavioral characters. Bootstrap support values deduced from 500 replicates and Bayesian posterior probabilities indicated under branches if ≥ 50.